Study of urinary estrogens profile in captive female sloth bears (*Melursus ursinus*) and influence of proximity to other bear gender groups

SYNOPSIS

For the Degree of

Doctor of Philosophy

ZOOLOGY

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Dayalbagh, Agra

2015

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1. INTRODUCTION:

Sloth bear

Kingdom: Animalia

Phylum: Chordata

Class: Mammalia

Order: Carnivora

Family: Ursidae

Genus: *Melursus*

Species : *ursinus*

Four species of bears are found in India viz. Sloth bear (*Melursus ursinus*), Himalayan Black bear (*Ursus thibetanus*), Brown bear (*Ursus arctos*) and Malayan Sun bear (*Helarctos malayanus*). Sloth bear (*Melursus ursinus*) is a nocturnal omnivorous species of bear found in the wild only in the Indian Subcontinent. Estimated population of the sloth bear is about 20,000 with less than 10,000 adults existing in the wild; and therefore it has been listed in IUCN Red List as Vulnerable. The wild population of sloth bears has been on the decline by 30 – 49% in last 30 years (IUCN Red List of Threatened Species, 2014).This decline can be attributed to the habitat loss and to some extent to the exploitation for body parts. Poaching and trade in sloth bears is also common in many parts of their range, including the capture and removal of cubs from the wild.

Understanding basic physiology of the species is fundamental in the efficient planning and execution of its conservation strategies. A combination of laboratory science with field techniques is essential to fight against extinction of a species. Information on the physiology of reproduction in the sloth bears is scanty and incoherent. Most of the records are from captive animals and are more from anecdotal evidences than from the scientific records.

The proposed study will provide critical new information hitherto unknown in sloth bears on the reproductive physiology and help towards better management of captive populations and sloth bear rehabilitation facilities.

2. BACKGROUND

Early study by Boone *et al* (1998) visually verified corpora lutea and measured serum progesterone concentrations in 8 semi-captive American black bears. Their data analysis suggested that this species of bears are induced ovulators. This was followed by the focus on the urinary estrogens and progestins profiles in female Giant Pandas, Indian rhinoceros and primates (Czekala *et al*. 2003; Stoops *et al.* 2004; Perret 2005). Studies on comparative reproductive seasonality in all Eight species of bears were also attempted (Spady *et al*. 2007), however, the reproductive hormones activities such as estrogen or progesterone in cycling sloth bear females were not dealt, and remain largely unknown. Such studies on the reproductive physiology in the other species of bear have shown the possibility of using the acute phase protein Ceruloplasmin as a non-invasive marker of the pregnancy status in Giant Panda (Willis *et al* 2011).

This study will be consequently the first attempt to establish the baseline data and standards for the urinary estrogen activities in the cycling female sloth bears by qualitative and quantitative analysis of urinary metabolites of this hormone and the influence of the proximity to different gender groups on these activities.

3. HYPOTHESIS

1. The urinary estrogen levels and thus the estrous in female sloth bears is influenced by the following factors
   a) Physical proximity to other females
   b) Physical proximity to males
   c) Olfactory / auditory / visual proximity to males without physical proximity

2. The quality and quantity of some proteins in the urine of a female sloth bear can be used as a marker for the characterization of estrous in the female sloth bears.

4. OBJECTIVES

- To study and analyze the urinary activity of estrogens in cycling female sloth bears
- To establish and provide a standard baseline reference for the same for further studies
- To study the effects of physical, visual, auditory and olfactory proximity to different gender groups on the activity of urinary estrogens
- To establish a reliable non-invasive method for characterization of reproductive status in the sloth bears
- To identify the urinary activity of some proteins in the cycling female sloth bears and establish their correlation with the estrous status of the animal.

5. PROTOCOLS

The site of the project will be Agra Bear Rescue Facility at Soor Sarovar Bird Sanctuary (27°15'15.3"N 77°51'10.8"E) as the facility houses about 230 sloth bears in 14 enclosures.

Phase I

- Individual reproductively cycling females will be selected for the study. The proximity and access of these individuals to other females and males in the same and neighboring enclosures will be recorded for phase II analysis.
- Urine samples will be collected from selected female bears. At least two samples will be collected per week from each individual. The samples will be collected from the floor of the den with sterile syringes. They will be immediately transferred to sterile containers, labeled and stored in a deep freezer at -20^{o}C.

- Behavioral data of the individual bears, including the estrous and mating behavior, will be recorded for the duration of samples collection.
- The samples will be then subjected to the Enzyme Immunoassay (EIA) or Radio Immunoassay (RIA) to measure the estrogens content of the samples.
- Qualitative and quantitative analysis of proteins will be carried out.
- From the results of the Estrogens assays, a baseline will be established for the urinary activity for estrogens for the study individuals in their current enclosure.
- This estrogen data will also identify which individual females are cycling, the duration of estrous, the inter-estrous interval, the effect of proximity to other females and to males on estrous cycling, and seasonal effects on estrous cycling.

Phase II

- Based on these results, in the following year the study individuals will be moved to enclosures with or without physical, visual, olfactory and auditory proximity to males.
- Urine samples will be collected, labeled and stored again as per the previously established protocol.
- These samples will be subjected again to estrogenassays to measure the estrogens.
- Qualitative and quantitative analysis of the urinary proteins identified in Phase I will be carried out

Model I: Datasheet for sample collection

Bear Name: Lilly
Age: 10 years

Date (d/m/y)	Time of urine collection	Quantity (ml)	Comments
23/04/2015	10.42 am	2	Nil
28/04/2015	11.15 am	3	Mixed with small amount of faeces
5/05/2015	9.45 am	2	Nil

Model II: Ethogram for reproductive behaviour observations

1. Non-reproductive behaviour

Self directed activities: Paw sucking, swaying, pacing, holding a part of self's body and playing

Interactive activities: Interacting with other bears (playing, fighting, touching, licking etc), playing with or using enrichments, walking around the enclosure, exploring natural and human-made objects in the enclosure, eating, drinking, interacting with the objects or animals or humans outside the enclosure through the fence

Passive activities: Lying, standing, sitting, sleeping

2. Reproductive behaviour

Mounting: Allowing the males to mount for copulation

Vocalization: Making grunting or wailing sounds for or during mating

Courtship: Following or showing interest in males for mating

Aggressiveness: Aggressive gesture (charging, display of canines, high pitched vocalization) towards other females and humans

Model III: Datasheet for behaviour record

Bear name: Lilly

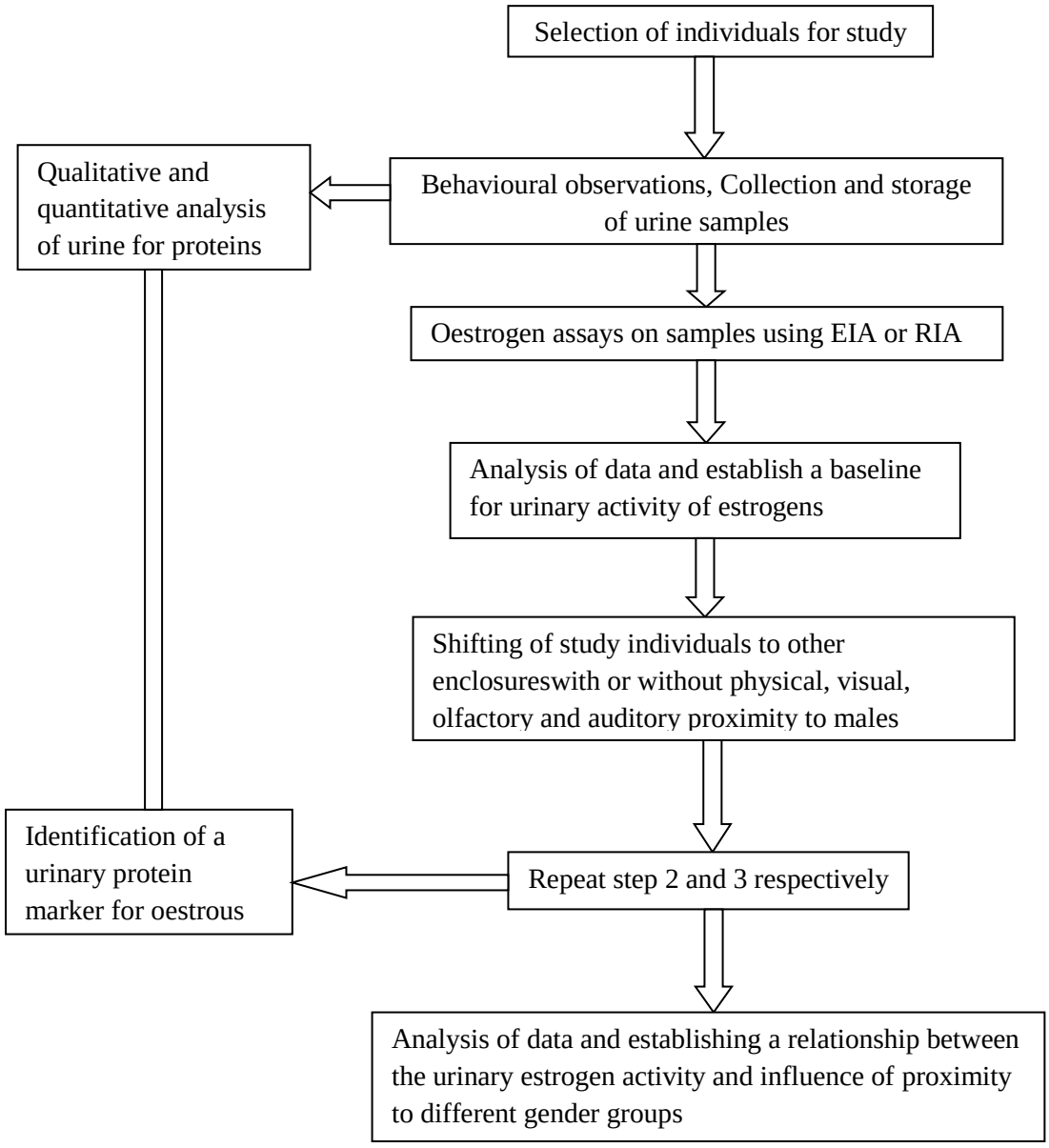
Age: 10 years

Date: 23/04/2015

Sampling event	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Non-Reproductive behaviour										
Self directed activities										
Interactive activities										
Passive activities										
Reproductive behaviour										
Mounting										
Vocalization										
Courtship										
Aggressiveness										

Observations are carried out for an hour. Each sampling event is after 6 minutes, thus giving 10 observations in an hour.

6. PLAN OF WORK


Selection of individuals for study
Behavioural observations, Collection and storage of urine samples
Qualitative and quantitative analysis of urine for proteins
Oestrogen assays on samples using EIA or RIA
Analysis of data and establish a baseline for urinary activity of estrogens
Shifting of study individuals to other enclosureswith or without physical, visual, olfactory and auditory proximity to males
Repeat step 2 and 3 respectively
Identification of a urinary protein marker for oestrous
Analysis of data and establishing a relationship between the urinary estrogen activity and influence of proximity to different gender groups

7. REFERENCES

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